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Masatoki Sato  
*Dartmouth College*

Haijing Li  
*Vanderbilt University*

Mine R. Ikizler  
*Vanderbilt University*

Jay A. Werkhaven  
*Vanderbilt University*

John V. Williams  
*Vanderbilt University*

*See next page for additional authors*

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**Authors**

Masatoki Sato, Haijing Li, Mine R. Ikizler, Jay A. Werkhaven, John V. Williams, James D. Chappell, Yi-Wei Tang, and Peter F. Wright

## NOTES

### Detection of Viruses in Human Adenoid Tissues by Use of Multiplex PCR<sup>∇</sup>

Masatoki Sato,<sup>1,5,6</sup> Haijing Li,<sup>2</sup> Mine R. Ikizler,<sup>1</sup> Jay A. Werkhaven,<sup>3</sup> John V. Williams,<sup>1</sup> James D. Chappell,<sup>1,4</sup> Yi-Wei Tang,<sup>2,4</sup> and Peter F. Wright<sup>1,4,6\*</sup>

*Departments of Pediatrics,<sup>1</sup> Medicine,<sup>2</sup> Otolaryngology,<sup>3</sup> and Pathology,<sup>4</sup> Vanderbilt University School of Medicine, Nashville, Tennessee 37232; Department of Pediatrics, Fukushima Medical University School of Medicine, Fukushima 960-1295, Japan<sup>5</sup>; and Department of Pediatrics, Dartmouth Medical School, Hanover, New Hampshire 03755<sup>6</sup>*

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**By PCR, we detected a high frequency of viruses in adenoids obtained from children without acute respiratory symptoms. Our results suggest that persistent/latent viral infection in the respiratory tract confounds interpretation of the association of pathogen detection by PCR with acute respiratory infection in these sources.**

Many aerobic and anaerobic bacteria form a well-recognized normal bacterial flora in the upper respiratory tract, and other bacteria, though potentially pathogenic, are often isolated not only from diseased but also from healthy individuals (2).

Prompted by the observation that primary human adenoid epithelial cell cultures used as a model system for studying respiratory viruses (23) sometimes show viral cytopathic effects, we investigated the presence of a wide variety of viruses in adenoid tissues. Herpesviruses, in particular Epstein-Barr virus (EBV), cytomegalovirus (CMV), human herpesvirus 6 (HHV-6), and HHV-7, have been detected in adenoid and tonsil tissues (1, 3, 5), and other studies have suggested that adenoids and/or tonsils can harbor these viruses latently (15, 16). In addition, it has been suggested that adenoviruses (ADVs) are harbored in latent form (7, 13). These viruses seem to form a “normal viral flora,” but little is known about whether any other viruses associated with an acute respiratory infection may become latent or persist for a long time in the respiratory tract after an acute infection. To clarify this matter, we performed PCR to detect viruses in adenoid tissues removed by adenoidectomy.

Thirty-five adenoids were obtained from children with a median age of 4 years after adenoidectomies were performed at Vanderbilt Children’s Hospital between May and December 2007 under a protocol approved by the Vanderbilt Institutional Review Board. The indications for adenoidectomy were determined by their primary care physicians in conjunction with the otolaryngologist performing the surgery. The most common indication was respiratory obstruction secondary to hypertrophy. The patients had no acute respiratory symptoms at the time of surgery.

Approximately 25 mg of each adenoid tissue specimen was used for DNA and RNA extraction using a QIAamp DNeasy tissue kit and a QIAamp RNeasy minikit (Qiagen, Valencia, CA), respectively. Eight monoplex PCR-enzyme immunoassays were used to detect herpes simplex virus (HSV), CMV, EBV, varicella-zoster virus (VZV), HHV-6, HHV-7, HHV-8, and ADVs, and a multiplex reverse transcriptase (RT)-PCR performed as previously described (11, 17, 18) was used to amplify influenza A virus (FLUAV), FLUBV, parainfluenza virus 1 (PIV-1), PIV-2, PIV-3, PIV-4, respiratory syncytial virus A (RSV-A), RSV-B, human metapneumovirus (hMPV), rhinoviruses (RhVs), and enteroviruses (EnVs) in a single reaction. RNA was also tested by real-time RT-PCR and conventional RT-PCR to detect human coronaviruses (hCoVs) and reoviruses (ReoVs), respectively, as previously described (6, 10, 19). Positive and negative controls were included with each run.

TABLE 1. Viruses detected in adenoid tissues from 35 patients

Virus(es)	No. (%) of positive specimens
ADV.....	28 (80.0)
HHV-7.....	18 (51.4)
EBV.....	15 (42.9)
EnVs.....	11 (31.4)
RhVs.....	6 (17.1)
HSV.....	1 (2.9)
CMV.....	1 (2.9)
HHV-6.....	1 (2.9)
PIV-1.....	1 (2.9)
VZV.....	0 (0)
HHV-8.....	0 (0)
PIV-2, -3, -4.....	0 (0)
FLUAV, FLUBV.....	0 (0)
RSV-A, -B.....	0 (0)
hMPV.....	0 (0)
hCoVs.....	0 (0)
ReoVs.....	0 (0)

\* Corresponding author. Mailing address: Dartmouth Medical School, 1 Medical Center Drive, Lebanon, New Hampshire 03756. Phone: (603) 653-6190. Fax: (603) 650-6199. E-mail: Peter.F.Wright@Dartmouth.edu.

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TABLE 2. Multiple viruses detected in adenoid tissues

No. of viruses detected (no. of samples)	Viruses detected	No. of samples with virus combination
Two (14)	HHV-7 + ADV	4
	ADV + EnV	3
	EBV + HHV-7	2
	EBV + EnV	2
	EBV + ADV	1
	ADV + RhV	1
	EnV + RhV	1
Three (10)	EBV + HHV-7 + ADV	5
	HHV-7 + ADV + EnV	2
	EBV + HHV-6 + RhV	1
	HHV-7 + ADV + RhV	1
	ADV + EnV + RhV	1
Four (4)	EBV + HHV-7 + ADV + EnV	1
	EBV + HHV-7 + ADV + RhV	1
	EBV + HHV-7 + ADV + PIV	1
	CMV + EBV + HHV-7 + ADV	1

At least one viral pathogen was detected in each of 35 adenoid tissues (Table 1). The viruses detected in greater than 15% of samples included ADVs, HHV-7, EBV, EnVs, and RhVs. Twenty-eight adenoids had multiple-virus-positive results (Table 2). In contrast, viruses not detected or rarely detected included VZV, HHV-8, HHV-6, CMV, HSV, hMPV, PIV-1, PIV-2, PIV-3, PIV-4, FLUAV, FLUBV, RSV-A, RSV-B, hCoV, and ReoVs.

This study represents the broadest panels of viruses examined in a large number of adenoids. Review of the published data shows some variation in the recovery of individual viruses (4, 12) but presents a consistent picture of the identification of viruses from adenoidal tissues.

The DNA and RNA viruses detected in this study were in lymphoid cells or in the complex epithelial layer overlying the organized adenoid lymphoid tissue. Of the DNA viruses, ADVs, EBV, and HHV-7 stood out as being commonly found. EBV and ADVs are recognized as causes of viral tonsillitis, and these viruses were detected frequently in our adenoids. EBV, in particular, is associated with more chronic disease in the respiratory lymphoid tissue (5, 15). The positive PCR results for EBV in 15 (43%) adenoids in the present study support latent infection of EBV in the adenoid tissue, though the high frequency of EBV in this mostly younger pediatric population is of interest, as EBV infection in the United States is most typically associated with young adults (14).

HHV-6 and HHV-7 are members of the *Betaherpesvirinae* subfamily. In the present study, HHV-7 was detected in 18 (51.4%) adenoids, but HHV-6 was detected in only 1 (2.9%) adenoid. This and previous studies suggest different latencies between HHV-6 and HHV-7 in adenoid tissue and saliva, in spite of the fact that HHV-7 is thought to occur later in life than HHV-6 (8, 9, 21, 22). Other DNA viruses that might have been expected in greater numbers, e.g., HSV and CMV, were rarely seen.

All of the classic RNA viruses associated with acute respiratory illness, FLUAV and FLUBV, RSV-A and RSV-B, PIV1-4, hCoV, and hMPV, were rarely found, suggesting little long-term carriage, though it should be noted that samples

were not obtained in the peak of the winter season for respiratory infections. In contrast, the picornaviruses, EnVs and RhVs, were seen in 31% and 17%, respectively, of tissues tested. We and others have recently demonstrated that RhVs from well children and adults are frequently identified by PCR (12, 20). The high frequency of EnVs was unexpected, as they are most commonly associated with enteric disease. Our results demonstrate that picornaviruses may be shed long term or cause a latent infection and/or suggest that EnVs may have a larger role in respiratory illness than previously thought. Little is known about ReoVs as causes of human disease. This study suggests that they are unlikely to cause chronic infections.

A caveat for using specimens from adenoidectomies or tonsillectomies is that they may be abnormal pathological specimens not representative of the general population. Elective surgery is usually postponed in the face of illness, and the majority of surgery is for obstructive sleep apnea, not recurrent infections (4).

Our results imply that viruses can be identified in adenoids and tonsils with regularity as "normal viral flora" and suggest that some respiratory viruses have more chronicity and hence a much less clear association with acute respiratory illness. We should consider the possibility that persistent/latent infections with many viral pathogens in the respiratory tract confound PCR diagnosis in a clinical setting and should use caution in interpreting findings from primary epithelial cells derived from these sources.

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